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DRUG EVALUATION IN THE PLASMODIUM FALCIPARUM-AOTUS MODEL

ANNUAL REPORT

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June 14, 1989

Supported by

U. S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick
Frederick, Maryland 21701-5012

Contract No. DAMD17-87-C-7163

Gorgas Memorial Institute of Tropical &
Preventive Medicine, Inc.
9650 Rockville Pike
Bethesda, MD 20814

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION: Unclassified	1b. RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3. DISTRIBUTION/AVAILABILITY OF REPORT: Approved for public release; distribution unlimited
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5. MONITORING ORGANIZATION REPORT NUMBER(S)
6a. NAME OF PERFORMING ORGANIZATION: Use same address as on cover; 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION
6c. ADDRESS (City, State, and ZIP Code)	7b. ADDRESS (City, State, and ZIP Code)
8a. NAME OF FUNDING/SPONSORING ORGANIZATION U.S.Army Medical Research & Development Command; 8b. OFFICE SYMBOL (If applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER: Contract No. DAMD17-87-C-7163
8c. ADDRESS (City, State, and ZIP Code): Ft. Detrick, Frederick, MD 21701-5012	10. SOURCE OF FUNDING NUMBERS: PROGRAM ELEMENT NO. 62770A; PROJECT NO. 3MI-62770A870; TASK NO. AJ; WORK UNIT ACCESSION NO. 003

11. TITLE (Include Security Classification)
Drug Evaluation in the Plasmodium falciparum - Aotus Model

12. PERSONAL AUTHOR(S)
Richard N. Rossan

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Annual	FROM 5/15/88 TO 5/14/89	1989 June 14	48

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	
06	13		Plasmodium falciparum; reversal of chloroquine resistance; Aotus lemurinus lemurinus; calcium channel blocker; artemisinin derivatives, RA1
06	15		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Blood-induced infections of the multidrug resistant Vietnam Smith/RE strain of Plasmodium falciparum in Aotus lemurinus lemurinus were used as a model for antimalarial drug evaluation studies. Trials to reverse chloroquine resistance in vivo with WR 256287, an analog of tiapamil , plus chloroquine, resulted only in suppression of parasitemia. Similar trials with WR 149244, desipramine, plus chloroquine, were partially successful in that primary treatments cleared parasitemias, but infections were not cured. Parasite clearance is the first indication that in vivo reversal of chloroquine-resistance may eventually be feasible in human patients. However, the toxicity of the combined drug regimen must be ameliorated.

Four derivatives of artemisinin, the active antimalarial principal of qinghao were evaluated for their antimalarial activity in the P. falciparum - Aotus model. Two of these drugs, WR 255131 (arteether) and WR 254986 (artemether),

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT: ☐ UNCLASSIFIED/UNLIMITED ☒ SAME AS RPT. ☐ DTIC USERS	21. ABSTRACT SECURITY CLASSIFICATION: UNCLASSIFIED	
22a. NAME OF RESPONSIBLE INDIVIDUAL: Mary Frances Bostian	22b. TELEPHONE (Include Area Code): 301-663-7325	22c. OFFICE SYMBOL: SGRD-RMI-S

DD Form 1473, JUN 86 — Previous editions are obsolete. — SECURITY CLASSIFICATION OF THIS PAGE

19. are oil soluble and administered intramuscularly in three doses, ranging from 0.25 to 64.0 mg/kg, q.12h. Arteether cleared parasitemias in a total of 23 of 25 treatments, and cured 16 of 24 infections. Artemether cleared parasitemias in a total of 20 of 23 treatments and cured 16 of 23 infections.

The two water soluble derivatives evaluated were WR 255663 (artelinate) and WR 256283 (artesunate). These drugs were administered intravenously or intramuscularly, at doses of 64.0 and 96.0 mg/kg (X3), q.6h, q.12h, or q.24h. Toxicity has been associated with these regimens. To date, artelinate cleared parasitemias in 14 of 15 treatments, and cured 4 of 15 infections. Artesunate cleared parasitemias in 3 of 3 treatments, and cured 1 of 3 infections.

SUMMARY

The objective of this contract is to evaluate experimental antimalarial drugs, alone or in combination, against experimentally induced trophozoite infections of Plasmodium falciparum in the Panamanian owl monkey (Aotus lemurinus lemurinus). For the studies reported herein, the Vietnam Smith/RE strain was used, resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine.

In vivo trials were continued to reverse chloroquine resistance in P. falciparum by the concomitant administration of a calcium channel blocker plus chloroquine. Initiation of such trials, summarized in the previous Annual Report for this contract, was based upon successful in vitro reversal of chloroquine resistance. The explanation of this phenomenon was based upon the hypothesis that the channel blocker prevents the active efflux of chloroquine by the parasite, allowing chloroquine to achieve a parasiticidal level.

Additional in vivo reversal trials with WR 256287, a structural analog of verapamil, administered orally 3X/day for seven days plus chloroquine for either 3 or 5 days, resulted in suppression of parasitemia only.

Desipramine (Norpramin), WR 149244, is a tricyclic psychotropic drug. Some drugs in this class have weak antimalarial activity and are calcium antagonists. Trials to reverse chloroquine-resistance in vivo with desipramine showed that a three day course of treatment with chloroquine cleared the parasitemia in 7 of 13 monkeys, but without infection cure. A seven day treatment course of desipramine plus chloroquine cleared parasitemia in 5 of 7 monkeys, and 2 of 5 infections were cured, but after repeat treatments. Seven monkeys died of drug toxicity. Although the combination of desipramine plus chloroquine reverses chloroquine resistance in vivo, at least to the extent of clearing primary parasitemias, its usefulness in human infections must be qualified until a non-toxic regimen is identified.

Four derivatives of artemisinin, the active antimalarial principal of the Chinese herb qinghao, were selected for evaluation in the P. falciparum-Aotus model. Two of these derivatives, WR 255131 (arteether), and WR 254986 (artemether), are oil soluble; WR 255663 (artelinate) and WR 256283 (artesunate) are water soluble.

Limited toxicity evaluation, based upon overt reaction and body weight gain or loss, indicated that a dose of 64.0 mg/kg (X3), IM, q.12h of arteether and artemether, was well-

tolerated by Aotus. Drug tolerance problems were associated with the intravenous administration of artelinate at a dose of 64.0 mg/kg (X3), q.6h, in a 30 mg/ml stock solution. Lower concentrations of stock solution, drug administration q.12h, and intramuscular injection of the drug, have reduced toxicity, but not entirely. Similar host tolerance difficulties were associated with artesunate, thus limiting the antimalarial evaluation.

For antimalarial evaluation, the two oil soluble derivatives, arteether and artemether, were administered intramuscularly q.12h, at doses ranging from 0.25 mg/kg (X3) to 64.0 mg/kg (X3), as both primary and repeat treatments. Arteether, WR 255131, cleared parasitemias in a total of 23 of 25 treatments, and cured 16 of 24 infections. Artemether, WR 254986, cleared parasitemias in a total of 20 of 23 treatments and cured 16 of 23 infections.

The two water soluble artemisinin derivatives, artelinate and artesunate were administered intravenously or intramuscularly, q.6h, q.12h, or q.24h. Doses were 64.0 and 96.0 mg/kg (X3). Artelinate, WR 255663, cleared parasitemias in a total of 14 of 15 treatments, and cured 4 of 15 infections. Artesunate, WR 256283, cleared parasitemias in a total of 3 of 3 treatments, and cured 1 of 3 infections.

The antimalarial activity of the two oil soluble artemisinin derivatives, arteether and artemether, are similar and both are more effective and less toxic than the water soluble derivatives, artelinate and artesunate.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources Commission of Life Sciences, National Research Council (NIH Publication No. 86-23, Revised 1985).

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EXPERIMENTAL PROCEDURES

The monkey-adapted Plasmodium falciparum strain, Vietnam Smith/RE (resistant to maximally tolerated doses of chloroquine, pyrimethamine, and quinine) was used to induce experimental malaria infections in Aotus lemurinus lemurinus for the evaluation of the antimalarial efficacy of candidate drugs. Infected blood, with sodium citrate (2.5%) as the anticoagulant, from untreated Aotus was diluted appropriately with chilled saline (0.85%), such that each milliliter contained 5,000,000 parasites, and this amount was injected into the saphenous vein of experimental and control monkeys.

Blood films, prepared and examined daily beginning on the first post-inoculation day, were stained with Giemsa. Parasitemias were evaluated as follows: negative, if no parasites were detected on a thick blood film after examination for at least 5 minutes; <10 parasites per cmm, if positive only on the thick blood film; parasite enumeration was by the Earle-Perez method and reported as the number of parasites per cmm.

Blood films from untreated Aotus, serving as passage and/or control subjects, were prepared and examined daily during the primary patent period, and daily thereafter for at least three consecutive days after parasites could last be detected on thick blood films. When parasitemia had cleared, films were made and examined twice weekly until a total of 100 negative days had been recorded. If a recrudescence occurred, blood films were obtained again on a daily basis.

The schema depicted in Figure 1 represents the design of a typical drug evaluation study. Parasitemias were evaluated daily during the treatment period and until blood films were negative for at least seven consecutive days. The frequency of smearing was then reduced to two times per week (Monday and Thursdays or Tuesdays and Fridays). If no recrudescences occurred during a 100 day examination period, the infection was considered to have been cured.

Drug doses were calculated as mg base per kg of body weight. Stock solution of water soluble compounds, at appropriate concentrations, were prepared with distilled water and stored at 8°C for the treatment period. If a compound was water insoluble, a suspension of the requisite amount of drug was prepared daily with 0.3% methylcellulose (in distilled water).

Oral administration of drugs was effected by gastric intubation with a 14 French catheter. The total amount of fluid administered, drug solution or suspension, and rinse was 14 ml.

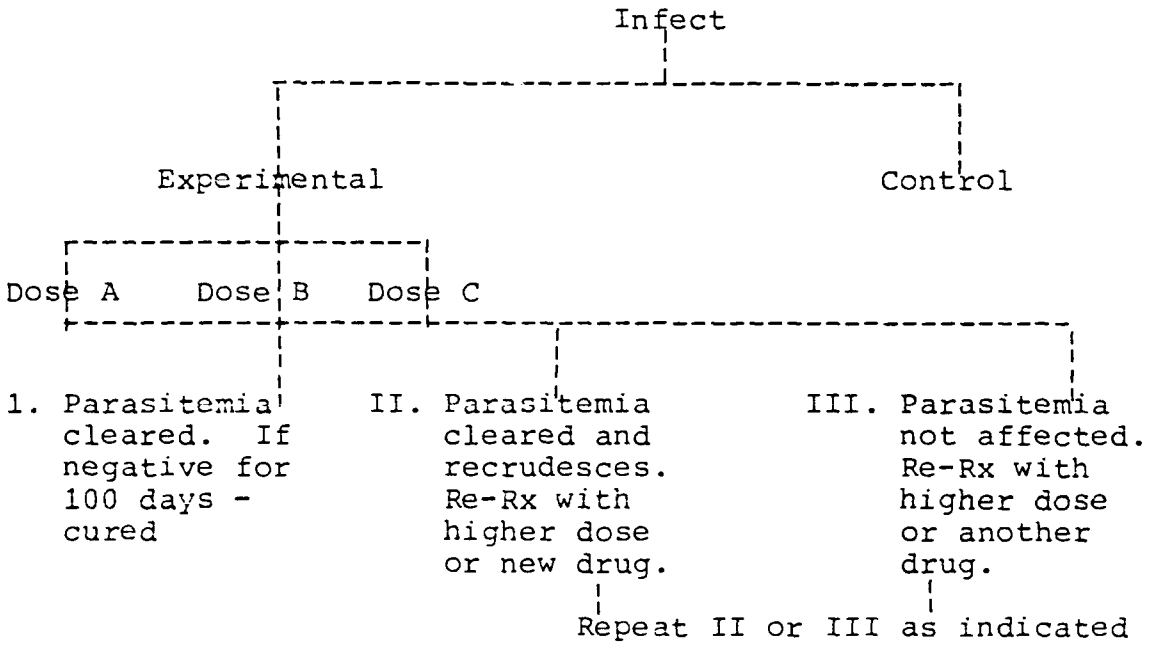
As indicated in the appropriate sections, some water soluble drugs were administered intravenously or intramuscularly; other water insoluble drugs were diluted in sesame oil and administered intramuscularly.

FIGURE 1

SCHEMA FOR DRUG EVALUATION AGAINST

PLASMODIUM FALCIPARUM

INDUCED INFECTIONS IN AOTUS LEMURINUS LEMURINUS



IN VIVO TRIALS TO REVERSE CHLOROQUINE RESISTANCE IN PLASMODIUM FALCIPARUM BY THE CONCOMITANT ADMINISTRATION OF CALCIUM CHANNEL BLOCKERS OR SIMILAR ACTING DRUGS

A. Introduction

Data associated with numerous trials to reverse chloroquine resistance in vivo were presented in a previous Annual Report (1). The genesis of these trials was based upon reports of in vitro reversal of chloroquine resistance in P. falciparum by verapamil (a calcium channel blocker) plus chloroquine (2, 3). Infections of the Vietnam Smith/RE strain of P. falciparum in Aotus were used for in vivo trials. In a total of 26 combined treatments during the primary patent period, suppression of parasitemia occurred in 17 monkeys. Verapamil plus chloroquine cured the infection in one monkey. In a total of 28 repeat treatments, infections were cured in 6 Aotus. The infection parameters in cured monkeys were identical to those of infected, untreated Aotus exhibiting self-cure, thus making it difficult to differentiate drug activity from acquired immunity.

Continuation of trials to reverse resistance to chloroquine are reported herein.

B. WR 256287AB (BN: BL 51153)

This Hoffman La Roche drug is a structural analog of tiapamil, related to verapamil. Although not as potent a calcium channel blocker in humans as verapamil, it was hoped that an analog without the cardiodynamic effects of verapamil might prove less toxic in combination with chloroquine. Also, WR 256287 is 4X more effective in vitro than verapamil in reversing chloroquine resistance. Previous in vivo trials (1) indicated that WR 256287 administered orally 3X/day for 7 days at a dose of 20.0 mg/kg plus chloroquine administered daily for three days significantly suppressed parasitemias of the chloroquine resistant Vietnam Smith/RE strain. Additional trials with this drug combination were undertaken.

Data presented in Tables 1 and 2 indicate that WR 256287 administered orally 3X/day for seven days at a dose of 20.0 mg/kg plus chloroquine (20.0 mg/kg, daily) for either 3 or 5 days suppressed parasitemias, but without parasite clearance. Some suppression of parasitemia was observed when chloroquine (20.0 mg/kg, daily) alone was administered.

C. WR 149244AD (BN: BL 54261)

WR 149244, desipramine (Norpramin), is a tricyclic psychotropic drug. Some drugs in this class have weak antimalarial activity and are calcium antagonists. In vitro reversal of chloroquine resistance in P. falciparum by desipramine was reported (4) at concentrations similar to those in patients treated for depression.

Data associated with trials of desipramine to reverse chloroquine resistance of P. falciparum infections in Aotus are detailed herein. As shown in Tables 3-6, WR 149244 was administered orally, once, twice, or three times daily, for either three or seven days. Neither desipramine nor chloroquine, administered alone, had significant antimalarial activity against parasitemias. A three-day course of treatment with desipramine plus chloroquine (Tables 3 and 5) cleared the parasitemias in 7 of 13 monkeys. Two of the seven treatments were primary, and no infections were cured. Additionally, six monkeys died within 2 to 3 days after initiating treatment with desipramine plus chloroquine.

The data presented in Tables 4 and 6 show that a 7-day course of treatment with desipramine plus chloroquine cleared parasitemias in 5 of 7 monkeys, and 2 of 5 infections were cured after repeat treatments. One Aotus died of drug toxicity on day 4 of treatment.

Overall, 13 of 24 (54.2%) parasitemias were cleared and 2 of 21 (8.3%) infections were cured (Table 7). A total of seven monkeys died of causes attributable to drug toxicity, i.e. a combination of desipramine plus chloroquine. An evaluation in uninfected Aotus of the acute toxicity of desipramine plus chloroquine, administered in different dose and regimens is indicated in Table 8. There were no deaths associated with these drug regimens.

D. CONCLUSIONS

The desideratum of *in vitro* chloroquine reversal is combined treatment with a calcium channel blocker plus chloroquine during the ascending phase of parasitemia resulting in parasite clearance and cure of infection. This sequence of events would be entirely the result of drug action. Infection cures subsequent to combined retreatment course may be attributable to both drug action and acquired immunity. The trials of *in vitro* reversal of chloroquine resistance presented in the previous Annual Report (1) showed that parasite clearance was not obtained with primary drug treatments, but only by repeat treatments.

Results of studies with desipramine plus chloroquine indicate that a primary drug treatment will clear parasitemias of the chloroquine-resistant Smith/RE strain of *P. falciparum*. Infection cures, however, were obtained only after repeat drug treatments. The potential use of desipramine plus chloroquine in human patients must be tempered by the toxic effects of this combination in *Aotus*. Additional drug evaluation in the monkey model may yield a non-toxic, curative regimen for appropriate use in patients infected with chloroquine-resistant malaria.

TABLE 1

DETAILED ACTIVITY OF WR 256287AB (BL 51153) PLUS WR 001544AB (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3
12351	20.0a 20.0b	2	40	20	18	8	4	3	8	105	Rx, different drug	
12352	20.0a 20.0b	2	35	20	33	28	57	87	212	468	Rx, different drug	
12356	20.0a 20.0c	4	34	26	57	87	128	25	30	74	Rx, different drug	
12437	20.0a 20.0c	1	32	6	14	2	0.3	<0.01	<0.01	0.08	Rx, different drug	
12439	20.0c	2	51	57	92	228	117	197	302	321	Rx, different drug	
12350	20.0b	1	33	43	24	22	75	43	434	265	Rx, different drug	

a WR 256287 3x/day
b Chloroquine 1x/day for 3 days
c Chloroquine 1x/day for 5 days

TABLE 2

SUMMARY OF THE ACTIVITY OF WR 256287AB (BL 51153) PLUS WR 001544AB (AR 20613), CHLOROQUINE, AGAINST INFECTION OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12351	20.0a 20.0b		+		n.a.	n.a.	Rx, different drug
12352	20.0a 20.0b		+		n.a.	n.a.	Rx, different drug
12356	20.0a 20.0c		+		n.a.	n.a.	Rx, different drug
12437	20.0a 20.0c		+		n.a.	n.a.	Rx, different drug
12349	20.0c		$\frac{+}{-}$		n.a.	n.a.	Rx, different drug
12350	20.0b		$\frac{+}{-}$		n.a.	n.a.	Rx, different drug

a WR 256287 3x/day

b Chloroquine 1x/day for 3 days

c Chloroquine 1x/day for 5 days

TABLE 3

DETAILED ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12349r	25.0a	321	665	107	DIED, malaria							
12351r	25.0a	105	80	136	81	95	72	Re-Rx				
12434	20.0b	79	82	542	369	345	Re-Rx					
12356r	1.0a 20.0b	74	55	96	25	Died, day 3 of Rx, possible drug toxicity						
12352r	4.0a 20.0b	468	165	468	120	18	2	0.2	<0.01	<0.01	<0.01	<0.01
12350r	8.0 20.0b	265	592	191	106	23	1	2	0.2	<0.01	<0.01	<0.01
12353r	8.0a 20.0b	65	15	5	1	1	1	6	13	30	Re-Rx	
12437r	16.0a 20.0b	0.08	0.8	3	1	0.2	<0.01	0	0	<0.01	<0.01	<0.01
12351rr	16.0a 20.0b	72	45	32	2	Died, day 3 of Rx, possible drug toxicity						
12107	25.0c 10.0b	4	9	47	29	61	382	58	166	Re-Rx		
12153	25.0c 10.0b	5	11	55	26	52	208	148	690	Re-Rx		
11093	25.0d 10.0b	4	7	65	25	104	111	209	241	Re-Rx		

TABLE 3 (CONT'D.)

DETAILED ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
11610	25.0d 10.0b	4	5	37	14	5	5	18	141	Re-Rx		
12423	25.0a 20.0b	55	63	Died, drug toxicity								
12422	25.0a 20.0b	48	33	209	72	0.08	Died, drug toxicity					
12447	25.0a 20.0b	53	56	197	Died, drug toxicity							
12353	25.0e 20.0b	1	22	10	0.3	0.09	<0.01	0	0	<0.01	<0.01	<0.01
12384	25.0e 20.0b	0.9	3	2	0.06	<0.01	0	0	0	0	0	0
12434r	25.0a 20.0b	345	527	204	Died, drug toxicity							
12446r	25.0d 10.0a	33	6	33	0.5	<0.01	<0.01	0	0	0	0	0
12384r	32.0a 20.0b	402	341	206	3	0.06	0	0	0	0	0	0
12353rr	32.0a 20.0b	30	37	10	0.1	0	0	0	0	0	0	0

TABLE 3 (CONT'D.)

DETAILED ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Daily Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7

a WR 149244, 3x/day

b Chloroquine, 1x/day

c WR 149244, 1x/day

d WR 149244, 2x/day

e WR 149244, initial dose 50.0 mg/kg, reduced to 25.0 mg/kg, 3x/day

TABLE 4

DETAILED ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment							Day Post Treatment			
			1	2	3	4	5	6	7	1	2	3	
11756	25.0a	35	35	191	104	272	Re-Rx						
12433	5.0b	61	75	241	266	315	Re-Rx						
12446	25.0a 5.0b	56	93	444	41	23	520	2	0.09	<0.01	<0.01	<0.01	Re-Rx
12433r	25.0a 20.0b	315	630	296	15	Died, drug toxicity							
11756r	25.0a 5.0b	272	269	90	249	110	197	45	9	0.7	1	0.4	Re-Rx
12153r	25.0c 10.0b	690	321	321	38	6	0.5	<0.01	0	0	0	0	
11093r	25.0c 10.0b	241	468	259	197	61	4	1	0.6	<0.01	0	0	
11610r	25.0c 10.0b	142	105	40	38	0.5	0.4	<0.01	<0.01	0	0	0	
12107r	25.0c 10.0b	166	135	112	42	22	2	0.6	<0.01	0	0	0	
11756rr	25.0c 10.0b	0.1	0.3	<0.01	0	0	0	0	0	0	0	0	

TABLE 4 (CONT.'D)

DETAILED ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Treatment							Day Post Treatment		
			1	2	3	4	5	6	7	1	2	3

a WR 149244, 3x/day

b Chloroquine, 1x/day

c WR 149244, 2x/day

TABLE 5

SUMMARY OF THE ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose X3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12349r	25.0a	+			n.a.	n.a.	Died, day 3 of Rx, malaria
12351r	25.0a		+		n.a.	n.a.	Re-Rx
12434	20.0b	+			n.a.	n.a.	Re-Rx
12356r	1.0a 20.0b		+		n.a.	n.a.	Died, day 3 of Rx, possible drug toxicity
12352r	4.0a 20.0b			+	13	34	
12350r	8.0a 20.0b			+	18	18	
12353r	8.0a 20.0b		+		n.a.	n.a.	Re-Rx
12437r	16.0 20.0b		+		n.a.	n.a.	
12351rr	16.0a 20.0b		+		n.a.	n.a.	Died, day 3 of Rx, possible drug toxicity
12107	25.0c 10.0b		+		n.a.	n.a.	Re-Rx
12153	25.0c 10.0b		+		n.a.	n.a.	Re-Rx
11093	25.0d 10.0b		+		n.a.	n.a.	Re-Rx
11610	25.0d 10.0b		+		n.a.	n.a.	Re-Rx

TABLE 5 (CONT.'D)

SUMMARY OF THE ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose X3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12423	25.0a 20.0b	+			n.a.	n.a.	Died, day 2 of Rx, drug toxicity
12422	25.0a 20.0b		+		n.a.	n.a.	Died, day 2 Post-Rx, drug toxicity
12447	25.0a 20.0b	+			n.a.	n.a.	Died, day 3 of Rx, drug toxicity
12353	25.0e 20.0b			+	6	5	Re-Rx
12384	25.0e 20.0b			+	5	11	Re-Rx
12434r	25.0a 20.0b	+			n.a.	n.a.	Died, day 3 of Rx, drug toxicity
12446r	25.0d 10.0a			+	6	26	
12384r	32.0a 20.0b			+	5	16	
12353rr	32.0a 20.0b			+	4	39	

a WR 149244, 3x/day
b Chloroquine 1x/day
c WR 149244, 1x/day
d WR 149244, 2x/day
e WR 149244, initial dose 50.0 mg/kg, reduced to 25.0 mg/kg, 3x/day

TABLE 6

SUMMARY OF THE ACTIVITY OF WR 149244AB (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 7 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
11756	25.0a	+			n.a.	n.a.	Re-Rx
12433	5.0b	+			n.a.	n.a.	Re-Rx
12446	25.0a 5.0b		+		n.a.	n.a.	Re-Rx
12433r	25.0a 20.0b		+		n.a.	n.a.	Died, day 4 of Rx, drug toxicity
11756r	25.0a 5.0b		+		n.a.	n.a.	Re-Rx
12153r	25.0c 10.0a			+	7	n.a.	Cured
11093r	25.0c 10.0a			+	8	12	
11610r	25.0c 10.0b			+	8	28	
12107r	25.0c 10.0b			+	8	12	
11756rr	25.0c 10.0b			+	3	n.a.	Cured

a WR 149244, 3x/day

b Chloroquine, 1x/day

c WR 149244, 2x/day

TABLE 7.

SUMMARY OF THE ACTIVITY OF WR 149244AD (BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM (AR 20613), CHLOROQUINE, AGAINST PLASMODIUM FALCIPARUM INFECTIONS

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Vietnam Smith/RE	225	75a			0/1	0/1	0/1	0/1
	300	75a	0/1	0/1			0/1	0/1
	20	5b	0/1	0/1			0/1	0/1
	60	20b	0/1	0/1			0/1	0/1
	36 60	12a 20b			1/1	0/1	1/1	0/1
	72 60	24a 20b			1/2	0/2	1/2	0/2
	75 30	25a 10b	0/2	0/2			0/2	0/2
	144 60	48a 20b			1/1	0/1	1/1	0/1
	150 30	50a 10b	0/2	0/2	1/1	0/1	1/3	0/3
	250 60	25a 20b	2/2	0/2			2/2	0/2
	288 60	96a 20b			2/2	0/2	2/2	0/2
	350 70	50a 10b			5/5	2/5	5/5	2/5
	525 35	75a 5b	0/1	0/1	0/1	0/1	0/2	0/2

a WR 149244
b Chloroquine

TABLE 8

EVALUATION OF ACUTE TOXICITY OF WR 149244AD
(BL 54261), DESIPRAMINE, IN COMBINATION WITH WR 001544BM
(AR 20613), CHLOROQUINE

Aotus No.	WR 149244 Dose mg/kg	X Daily	No. Days	WR 001544 Dose mg/kg	X Daily	No. Days	Body Wt.gms Pre-Rx	Post-Rx
11773	25.0	1	3	10.0	1	3	824	828
11453	25.0	2	3	10.0	1	3	836	828
12150	5.0	1	7	10.0	1	7	774	795
11373	10.0	1	7	10.0	1	7	885	864
11476	25.0	1	7	10.0	1	7	779	784
11475	5.0	2	7	10.0	1	7	860	829
12151	10.0	2	7	10.0	1	7	807	795
11775	25.0	2	7	10.0	1	7	858	869

COMPARISON OF THE ANTIMALARIAL EFFICACY OF FOUR ARTEMISININ DERIVATIVES IN THE PLASMODIUM FALCIPARUM - AOTUS MODEL

A. INTRODUCTION

An herb, qinghao (Artemisia annua L.), has been used in China for more than 400 years against the chills and fever of malaria (5). The active antimalarial principal of the herb has been identified as a 15-carbon sesquiterpene lactone endoperoxide and named artemisin. Studies in China with patients infected with P. falciparum or P. vivax showed that artemisin, an oil soluble derivative (artemether), and a water soluble derivative (artesunate) possessed significant antimalarial activity. Synthesis and selection of new artemisin derivatives yielded an oil soluble ethyl ether derivative, arteether, and a water soluble derivative, sodium artelinate. These two newly synthesized derivatives, and artemether and artesunate were selected for comparison of their antimalarial efficacy against infections of the multi-drug resistant Vietnam Smith/RE strain of P. falciparum in Aotus. Subsequent sections will delineate these studies.

All drugs, sesame oil, and sodium bicarbonate, were provided by the Division of Experimental Therapeutics, Walter Reed Army Medical Center.

B. Limited toxicity evaluation of four artemisinin derivatives

Since no studies with these drugs have been done in Aotus, it was considered necessary to evaluate the toxicity of at least the highest projected dose in this monkey. Animals cured of a malarial infection were used and drug toxicity was monitored by body weight, and overt symptoms.

1. WR 255131AE (BN: BL 48816), arteether
   WR 254986AB (BN: BL 26767), artemether

   Each of these compounds, soluble in sesame oil, were administered at a dose of 64.0 mg/kg (IM)X3, q.12h. The data presented in Table 9 show that the monkey (12007) administered WR 255131, arteether evidenced some loss of body weight beginning 14 days post-treatment, but that one month post-treatment the pre-treatment body weight had been regained. The body weight loss in Aotus 12294, administered WR 254986, artemether, was attributed to an intestinal amoeba infection. No drug toxicity was associated with artemether, per se.

2. WR 255663AG (BN: BL 54038)
   WR 255663AH (BN: BL 55866), sodium artelinate

   This water soluble artemisin derivative, sodium artelinate, was administered intravenously. The first monkey to receive artelinate was administered a dose of 64.0 mg/kg at 8:00 AM, using a 30 mg/ml stock solution. Following the second and third doses, each at 6 hour intervals, the animal became hypotonic, a condition that persisted for about 10 minutes. The monkey died of drug toxicity on day 2 post treatment. It was suggested that the concentration of the stock solution may have contributed to the toxic reaction, causing a precipitation of the drug in the vascular system. Subsequent concentrations of stock solutions of artelinate were reduced to either 10 mg per ml or 15 mg per ml. As shown in Table 10, Aotus 11805 was administered a 64.0 mg/kg (X3), q.6h of artelinate. Hypotonia was again noted after doses 2 and 3. A loss of body weight was noted beginning on day 6 post treatment, but the pre-treatment body weight was regained by day 26 post treatment.

   Results of an additional toxicity evaluation of artelinate are presented in Table 11. Doses of 4.0, 16.0, 32.0, and 64.0 mg/kg (X3), IV, q.6h, were administered to a total of four Aotus. During a four-month post treatment observation period, the lowest

body weight loss was associated with the 4.0 mg/kg dose, while the monkey experiencing the highest mean body weight loss received the 16.0 mg/kg dose. There was, however, no overt manifestation of drug toxicity.

3. WR 256283AA (BN: BL 28556), artesunate

Sodium artesunate, a water soluble artemisin derivative, was converted to artesunic acid, 10 minutes before intravenous administration, by the addition of sodium bicarbonate (5% solution). As shown in Table 10, one monkey was administered a 64.0 mg/kg X3 dose, q.6h, for toxicity evaluation. Some body loss (about 2%) occurred between days 8 and 16 post treatment, with no other adverse symptoms. Problems associated with administration of artesunic acid will be indicated in a subsequent section of this report.

TABLE 9

TOXICITY EVALUATION OF WR 255131AE (BL 48816), ARTEETHER, AND WR 254986AB (BL 26767), ARTEMETHER

Aotus No.	Drug, Dose, Notes	Days Post-Rx	Body Weight-gms
12007	WR 255131AE, 64.0 mg/kg (x3), IM, q. 12h	-2	849
		2	814
		5	822
		8	844
		11	843
		14	814
		16	825
		19	828
		22	818
		29	840
12294	WR 254986AB, 64.0 mg/kg (x3), IM, q. 12h	-2	725
		2	715
		5	710
		8	707
		11	707
		14	686
		16	677
	Intestinal amoebae Rx Tinidizol, 250 mg/kg for 3 days	19	670
		22	677
		29	677

TABLE 10

TOXICITY EVALUATION OF WR 255663AG (BL 54038), SODIUM ARTELINATE AND WR 256283AA (BL 28556), SODIUM ARTESUNATE

Aotus No.	Drug, Dose, Notes	Days Post-Rx	Body Weight-gms
11805	WR 255663AG 64.0 mg/kg (x3), IV, q. 6h. Monkey became flaccid after administration of doses 2 and 3	-2	773
		2	795
		4	790
		6	755
		8	731
		10	718
		12	719
		14	731
		26	774
11806	WR 256283AA, 64.0 mg/kg (x3), IV, q.6h.	-2	879
		2	866
		4	866
		6	870
		8	856
		10	857
		12	860
		14	853
		26	871

TABLE 11

FURTHER EVALUATION OF THE TOXICITY OF
WR 255663AG (BL 54038), SODIUM ARTELINATE

BODY WEIGHT (GMS)				
	MONKEY NO.			
DAYS POST Rx	12324	11972	12316	11335
-5	797	934	870	898
0 Rx	4.0 mg/kg*	16.0 mg/kg*	32.0 mg/kg*	64.0 mg/kg*
2	800	920	848	877
5	790	904	849	877
8	785	893	829	843
11	788	872	821	847
14	799	849	820	859
17	774	823	792	823
20	780	811	800	844
23	754	805	789	831
36	782	759	805	852
121	801	796	874	815
mean body weight loss	12	91	47	51

* (x3), IV, q.6h

C. Antimalarial activity of four artemisinin derivatives

1. WR 255131AE (BN: BL 48816), arteether

Evaluation of arteether against infections of the Vietnam Smith/RE strain of P. falciparum is detailed in Table 12 and summarized in Table 13. Three doses of the drug were administered intramuscularly at 8:00 AM, 8:00 PM, and 8:00 AM. A dose of 0.25 mg/kg (X3) suppressed parasitemia in each of two Aotus. Two primary treatments and two retreatments at a dose of 1.0 mg/kg (X3) cleared parasitemia in 4 of 4 monkeys. The infection in 1 of 4 monkeys may be cured.

Parasitemias in nine Aotus were cleared with a dose of 4.0 mg/kg (X3); infections were cured in two monkeys, recrudescence occurred in three monkeys, and post treatment observation is continuing in four Aotus.

A dose of 16.0 mg/kg (X3) cured the infection in 4 of 4 monkeys; the curative activity of retreatment with this dose has not been determined in one Aotus. Parasitemias were cleared in 5 of 5 monkeys with a dose of 64.0 mg/kg (X3), and infections were cured in four of these animals. The fifth monkey died on day 51 post-treatment of gastric dilatation, which was not considered attributable to arteether.

2. WR 254986AB (BN: BL 26767), artemether

The detailed antimalarial activity of artemether against Vietnam Smith/RE infections is shown in Table 14 and summarized in Table 15. Parasitemias were suppressed in each of two Aotus administered a dose of 0.25 mg/kg (X3).

A dose of 1.0 mg/kg (X3) suppressed primary parasitemia in one Aotus and cleared parasitemia in one monkey; retreatment with this dose cleared parasitemia in 2 of 2 Aotus. Infection cure remains to be determined in one monkey.

In nine Aotus administered a dose of 4.0 mg/kg (X3), the infection was cured, to date, in 2 of 6 monkeys following primary treatment. Post-treatment examination is continuing in five Aotus.

Infections were cured in 4 of 4 monkeys with a dose of 16.0 mg/kg (X3), and 5 of 5 monkeys with a dose of 64.0 mg/kg (X3).

3. WR 255663AG (BN: BL 54038)
WR 255663AH (BN: BL 55866), artelinate

Two drug lots of sodium artelinate were used for antimalarial evaluation, as detailed in Table 16 and summarized in Table 17. Four Aotus, infected with the Vietnam Smith/RE strain of P. falciparum, received a 64.0 mg/kg (X3) dose, administered intravenously, q.6h. The parasitemia was cleared (with recrudescence) in one monkey, suppression of parasitemia resulted in one animal, and two monkeys died of drug toxicity, on day 1 and day 6 post treatment, respectively. A 64.0 mg/kg (X3) dose, administered intravenously q.12h, cleared the parasitemia in 2 of 2 Aotus, but the infection was not cured.

Intramuscular administration of a dose of 64.0 mg/kg (X3), q.12h cleared the parasitemia in 2 of 2 monkeys; however, the infections were not cured. Five monkeys were administered intravenously a 64.0 mg/kg (X3) dose of artelinate, q.24h. The parasitemia was cleared in four of these Aotus; one animal died of drug toxicity on day 2 post treatment. The infection was cured in 1 of 4 treated monkeys. Retreatment with a dose of 96.0 mg/kg (X3) was as follows: two animals administered the drug intravenously, q.12h, with parasite clearance in both, and cured the infection in one monkey; two subjects received the drug intramuscularly, q.12h, resulting in parasite clearance and blood films remain negative for > 57 days; this dose, administered intravenously q.24h to one monkey, cleared the parasitemia, with recrudescence. The highest dose evaluated in this study, 128.0 mg/kg (X3), intravenously, q.6h, was toxic as the monkey died on day 2 post treatment.

4. WR 256283AA (BN: BL 28556)
WR 256283AB (BN: BL 35613), artesunate

Results of pilot evaluation studies with artesunate (two drug lots) are shown in Table 18 and 19. Intravenous adminstration (q.6h) of 64.0 mg/kg (X3) of artesunate suppressed parasitemia in one monkey, that died of an intercurrent infection on day 9 post treatment. A dose of 64.0 mg/kg (X3) administered intravenously q.12h cleared the parasitemia, with recrudescence, in one monkey; one animal died of drug toxicity on day 1 post treatment. Intramuscular administration (q.12h) of 64.0 mg/kg (X3) cleared parasitemia in one Aotus (with probable infection cure) and one monkey died of drug toxicity.

A 96.0 mg/kg (X3) dose administered intramuscularly (q.12h) cleared parasitemia in one monkey, but did not cure the infection.

D. CONCLUSIONS

Limited toxicity evaluation of four artemisinin derivatives show that the two oil soluble derivatives, arteether and artemether, are well tolerated following intramuscular administration in Aotus. Intravenous administration of the water soluble derivative, sodium artelinate, is tolerated when the stock solution concentration is 10 to 15 mg/ml. Toxicity problems associated with sodium artesunate, administered as artesunic acid, remain to be resolved.

Antimalarial evaluation of arteether and artemether, against infections of the multi-drug resistant Vietnam Smith/RE strain of P. falciparum indicate a similar activity by both drugs. Both drugs clear parasitemias when administered at doses of $\geq$ 1.0 mg/kg (X3), and $\geq$ 66% of the infections are cured with doses of $\geq$ 4.0 mg/kg (X3).

Primary or repeat treatments with artelinate or artesunate, at doses of 64.0 or 96.0 mg/kg (X3) cleared parasitemias, but only 27% of the infections were cured. Neither of the water soluble artemisinin derivatives is as effective as the oil soluble derivatives, arteether or artemether.

TABLE 12

DETAILED ACTIVITY OF WR 255131AE (BL 48816), ARTEETHER AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Rx		Day Post Treatment							
			1	2	1	2	3	4	5	6	7	8
12449	0.25	4	111	37	130	22	2	0.7	2	8	111	Re-Rx
12472	0.25	1	86	57	18	1	<0.01	<0.01	<0.01	0.2	2	Re-Rx
12470	1.0	1	68	65	1	<0.01	<0.01	<0.01	0	0	0	0
12473	1.0	1	70	51	1	<0.01	<0.01	<0.01	0	0	0	0
12449r	1.0	8	111	74	1	0.1	0.06	<0.01	0	0	0	0
12472r	1.0	0.2	2	9	0.7	<0.01	<0.01	<0.01	0	0	0	0
12410	4.0	11	56	55	2	0.3	<0.01	<0.01	0	0	0	0
12362	4.0	16	34	20	1	0.1	<0.01	<0.01	0	0	0	0
12363	4.0	13	33	33	0.6	0.06	<0.01	<0.01	0	0	0	0
12367	4.0	11	34	61	1	0.3	<0.01	<0.01	<0.01	0	0	0
12471	4.0	1	18	6	0.2	<0.01	<0.01	<0.01	0	0	0	0
12474	4.0	1	117	45	0.9	<0.01	<0.01	<0.01	0	0	0	0
12470r	4.0	79	161	53	0.1	<0.01	<0.01	<0.01	0	0	0	0
12449rr	4.0	0.01	0.01	0.01	0	0	0	0	0	0	0	0
12472rr	4.0	39	90	8	0.01	0	0	0	0	0	0	0
12442	16.0	28	40	23	0.2	<0.01	<0.01	<0.01	0	0	0	0
12359	16.0	17	40	25	5	4	<0.01	<0.01	0	0	<0.01	0
12360	16.0	27	142	42	40	6	0.2	<0.01	<0.01	<0.01	0	<0.01
12412	16.0	9	74	35	2	0.4	<0.01	<0.01	0	0	0	<0.01
12474r	16.0	142	126	30	1	<0.01	<0.01	<0.01	<0.01	0	0	0
12354	64.0	18	32	11	1	0.2	<0.01	<0.01	0	0	0	0
12366	64.0	16	21	10	0.1	0.2	<0.01	<0.01	<0.01	0	0	0
12400	64.0	14	68	20	0.2	0.09	<0.01	<0.01	0	0	0	0
12413	64.0	10	105	60	3	0.2	<0.01	<0.01	0	0	0	<0.01
12424	64.0	14	228	149	80	42	25	7	0.3	<0.01	0	0

* Three doses administered intramuscularly q.12h

TABLE 13

SUMMARY OF THE ACTIVITY OF WR 255131AE (BL 48816), ARTEETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12449	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12472	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12470	1.0			+	7	15	Re-Rx, higher dose
12473	1.0			+	7		Negative > 43 days
12449r	1.0			+	7	19	Re-Rx, higher dose
12472r	1.0			+	7	19	Re-Rx, higher dose
12410	4.0			+	7	18	Rx, WR 255663
12362	4.0			+	7	n.a.	Cured
12363	4.0			+	7	n.a.	Cured
12367	4.0			+	12	19	Rx, WR 255663
12471	4.0			+	7		Negative > 43 days
12474	4.0			+	7	14	Re-Rx, higher dose
12470r	4.0			+	7		Negative > 22 days
12449rr	4.0			+	3		Negative > 13 days
12472rr	4.0			+	4		Negative > 12 days
12442	16.0			+	7	n.a.	Cured
12359	16.0			+	10	n.a.	Cured
12360	16.0			+	11	n.a.	Cured
12412	16.0			+	7	n.a.	Cured
12474r	16.0			+	7		Negative > 21 days
12354	64.0			+	7	n.a.	Cured
12366	64.0			+	12	n.a.	Died Day 51 Post-Rx, gastric dilatation
12400	64.0			+	7	n.a.	Cured
12413	64.0			+	7	n.a.	Cured
12424	64.0			+	9	n.a.	Cured

TABLE 14

DETAILED ACTIVITY OF WR 254986AB (BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Rx		Day Post Treatment						
			1	2	1	2	3	4	5	6	7	8
12428	0.25	3	114	67	8	2	0.4	<0.01	<0.01	0.1	9	Re-Rx
12482	0.25	0.9	96	25	0.4	<0.01	<0.01	<0.01	0	0	<0.01	<0.01
12421	1.0	5	74	83	23	2	0.7	<0.01	<0.01	<0.01	0	0
12476	1.0	1	56	20	1	<0.01	<0.01	<0.01	0	0	0	0
12428r	1.0	0.1	9	3	0.2	0.09	<0.01	0	0	0	0	0
12482r	1.0	582	117	136	2	0.09	0.1	<0.01	<0.01	0	0	0
12390	4.0	15	20	19	1	0.6	<0.01	<0.01	0	0	0	<0.01
12398	4.0	35	28	6	0.1	0.04	<0.01	<0.01	0	0	0	0
12414	4.0	13	44	3	0.08	<0.01	<0.01	<0.01	0	0	0	0
12415	4.0	13	21	20	0.1	0.1	0	<0.01	0	0	0	0
12430	4.0	8	140	48	28	1	<0.01	<0.01	<0.01	0	0	0
12453	4.0	2	57	26	8	0.1	<0.01	<0.01	0	0	0	0
12421r	4.0	1	2	0.1	<0.01	0	0	0	0	0	0	0
12476r	4.0	14	111	38	4	5	2	<0.01	<0.01	0	0	0
12166	16.0	20	43	17	0.8	0.2	<0.01	<0.01	0	0	0	0
12388	16.0	18	26	18	0.8	0.1	<0.01	<0.01	0	0	<0.01	0
12401	16.0	16	35	21	0.2	0.1	<0.01	<0.01	0	0	0	<0.01
12403	16.0	23	65	53	0.5	0.2	<0.01	<0.01	0	0	0	0
12393	64.0	21	48	16	0.1	0.2	<0.01	<0.01	0	0	<0.01	<0.01
12399	64.0	29	30	15	0.2	0.07	<0.01	<0.01	<0.01	<0.01	0	<0.01
12402	64.0	39	60	43	0.3	0.04	<0.01	<0.01	0	0	0	<0.01
12404	64.0	30	49	5	0.07	0.06	<0.01	<0.01	0	0	0	<0.01
12425	64.0	11	252	129	49	34	32	5	0.6	<0.01	0	0

* 3 doses administered intramuscularly, q.12h

TABLE 15

SUMMARY OF THE ACTIVITY OF WR 254986AB (BL 26767), ARTEMETHER, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12428	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12482	0.25		+		n.a.	n.a.	Re-Rx, higher dose
12421	1.0		+		n.a.	n.a.	Re-Rx, higher dose
12476	1.0			+	7	15	Re-Rx, higher dose
12428r	1.0			+	6		Negative > 35 days
12482r	1.0			+	8	28	Re-Rx, higher dose
12390	4.0			+	7	19	Rx, WR 255663
12398	4.0			+	7	n.a.	Cured
12414	4.0			+	7	n.a.	Cured
12415	4.0			+	7	25	
12430	4.0			+	8		Negative > 42 days
12453	4.0			+	7		Negative > 43 days
12421r	4.0			+	4		Negative > 32 days
12476r	4.0			+	8		Negative > 21 days
12482rr	4.0						In progress
12166	16.0			+	7	n.a.	Cured
12388	16.0			+	12	n.a.	Cured
12401	16.0			+	7	n.a.	Cured
12403	16.0			+	7	n.a.	Cured
12393	64.0			+	11	n.a.	Cured
12399	64.0			+	11	n.a.	Cured
12402	64.0			+	11	n.a.	Cured
12404	64.0			+	7	n.a.	Cured
12425	64.0			+	8	n.a.	Cured

TABLE 16

PILOT EVALUATION: DETAILED ACTIVITY OF WR 255663AG/AH (BL 54038/BL 55866), ARTELINATE, AGAINST INFECTION OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3										
		Day Pre-Rx	Day of Treatment			Day Post Treatment						
			1	2	3	1	2	3	4	5	6	7
12431	64.0	12	160			74	Died, drug toxicity					
12093	64.0a	7	36			11	0.4	<0.01	<0.01	<0.01	<0.01	<0.01
12234	64.0a	20	35			9	1	0.1	<0.01	<0.01	0	0
12383	64.0a	5	27			20	0.4	0.07	<0.01	<0.01	Died, drug toxicity	
12035	64.0b	26	30	9		9	2	0.6	<0.01	<0.01	<0.01	0
12406	64.0b	14	27	11		0.1	0.04	<0.01	0	0	0	0
12191	64.0c	1	18	0.7		<0.01	<0.01	0	0	0	0	0
12200	64.0c	9	40	1		0.3	<0.01	0	0	0	0	0
12379	64.0a	8	22	1	0.1	<0.01	<0.01	0	0	0	0	0
12380	64.0d	13	55	2	<0.01	<0.01	Died, drug toxicity					
12367r	64.0d	351	228	76	1	<0.01	0	0	0	0	0	0
12390r	64.0d	111	197	53	6	0.2	<0.01	0	0	0	0	0
12410r	64.0d	80	265	42	0.5	0.05	<0.01	0	0	0	0	0
12234r	96.0b	234	296	40		0.3	<0.01	0	0	0	0	0
12406r	96.0b	148	409	69		1	0.07	<0.01	0	0	0	0
12191r	96.0c	2	5	2		0.5	<0.01	<0.01	0	0	0	0
12379r	96.0d	1	4	0.5	<0.01	0	0	0	0	0	0	0
12200r	96.0c	444	419	89		2	1	<0.01	0	0	0	0
12093r	128.0a	3	3			1 Died; drug toxicity						

a IV, q.6h
b IV, q.12h
c IM,q.12h
d IV, q.24h

TABLE 17

PILOT EVALUATION: SUMMARY OF THE ACTIVITY OF WR 255663AG/AH (BL 54038/BL 55866), ARTELINATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recru-descence	Notes
		None	Suppressed	Cleared			
12431	64.0a	+			n.a.	n.a.	Died, day 2 Post-Rx, drug toxicity
12093	64.0a		+		n.a.	n.a.	Re-Rx, higher dose
12234	64.0a			+	7	9	Re-Rx, higher dose
12383	64.0a		+		n.a.	n.a.	Died, day 6 post-Rx, drug toxicity
12035	64.0b			+	9	14	
12406	64.0b			+	6	9	Re-Rx, higher dose
12191	64.0c			+	5	15	Re-Rx, higher dose
12200	64.0c			+	5	11	Re-Rx, higher dose
12379	64.0d			+	6	22	Re-Rx, higher dose
12380	64.0d		+		n.a.	n.a.	Died, day 2 Post-Rx, drug toxicity
12367r	64.0d			+	5	20	
12390r	64.0d			+	6	13	
12410r	64.0d			+	6	n.a.	Cured
12234r	96.0b			+	6	n.a.	Cured
12406r	96.0b			+	6	20	
12191r	96.0c			+	6		Negative > 57 days
12397r	96.0d			+	4	16	
12200r	96.0c			+	6		Negative > 57 days
12093r	128.0a	+			n.a.	n.a.	Died, day 2 post-Rx, drug toxicity

a IV, q.6h
b IV, q.12h
c IM, q.12h
d IV, q.12h

TABLE 18

PILOT EVALUATION: DETAILED ACTIVITY OF WR 256283AA/AB (BL 28556/BL 35613), ARTESUNATE, AGAINST INFECTION OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Aotus No.	Dose Mg/Kg	Parasitemia per cmm x 10^3									
		Day Pre-Rx	Day of Rx		Day Post Treatment						
			1	2	1	2	3	4	5	6	7	8
12429	64.0a	8	147	99	99	29	27	7	0.8	<0.01	.01	DIED*
12381	64.0b	5	17	0.3	<0.01	<0.01	0	0	0	0	0	0
12417	64.0b	5	71	19	DIED, drug toxicity							
12386	64.0c	6	28	0.7	<0.01	<0.01	0	0	0	0	0	0
12387	64.0c	7	65	2	0.2	<0.01	0	DIED, drug toxicity				
12381r	96.0b	2	0.6	1	<0.01	0	0	0	0	0	0	0

a IVX3, q.6h
b IVX3, q.12h
c IMX3, q.12h
* Intercurrent infection

TABLE 19

PILOT EVALUATION: SUMMARY OF THE ACTIVITY OF WR 256283AA/AB (BL 28556/BL 35613), ARTESUNATE, AGAINST INFECTIONS OF THE VIETNAM SMITH/RE STRAIN OF PLASMODIUM FALCIPARUM

Monkey No.	Dose x 3 Mg/Kg	Response of Parasitemia to Rx			Days from Initial Rx to Parasite Clearance	Days from Final Rx To Recrudescence	Notes
		None	Suppressed	Cleared			
12429	64.0a		+		n.a.	n.a.	Died, day 9 Post-Rx, intercurrent infection
12381	64.0b			+	5	16	Re-Rx, higher dose
12417	64.0b	+			n.a.	n.a.	Died, day 1 Post-Rx*
12386	64.0c			+	5		Negative > 80 days
12387	64.0c		+		n.a.	n.a.	Died, day 4 Post-Rx*
12381r	96.0b			+	4	17	

a IV, q.6h
b IV, q.12h
c IM, q.12h

* Drug toxicity

TABLE 20

ACTIVITY OF FOUR ARTEMISININ DERIVATIVES
AGAINST INFECTIONS OF PLASMODIUM FALCIPARUM

MALARIA STRAIN	DOSE mg/kg TOTAL	DOSE mg/kg DAILY	PRIMARY TREATMENTS CLEARED	PRIMARY TREATMENTS CURED	REPEAT TREATMENTS CLEARED	REPEAT TREATMENTS CURED	TOTAL TREATMENTS CLEARED	TOTAL TREATMENTS CURED
Vietnam Smith/RE			WR 255131AE (BL 48816), arteether					
	0.75	0.25	0/2	0/2			0/2	0/2
	3.0	1.0	2/2	1/2(?)	2/2	0/2	4/4	1/4(?)
	12.0	4.0	6/6	3/6(?)	3/3	3/3(?)	9/9	6/9(?)
	48.0	16.0	4/4	4/4	1/1	1/1(?)	5/5	5/5(?)
	192.0	64.0	5/5	4/4			5/5	4/4
			WR 254986AB (BL 26767), artemether					
	0.75	0.25	0/2	0/2			0/2	0/2
	3.0	1.0	1/2	0/2	2/2	1/2(?)	3/4	1/4(?)
	12.0	4.0	6/6	4/6(?)	2/2	2/2(?)	8/8	6/8(?)
	48.0	16.0	4/4	4/4			4/4	4/4
	192.0	64.0	5/5	5/5			5/5	5/5
			WR 255663AG/AH (BL 54038/BL 55866), artelinate					
	192.0	64.0	6/7	0/7	3/3	1/3	9/10	1/10
	288.0	96.0			5/5	3/5(?)	5/5	3/5(?)
			WR 256283AA/AB (BL 28556/BL 35613), artesunate					
	192.0	64.0	2/2	1/2(?)			2/2	1/2(?)
	288.0	96.0			1/1	0/1	1/1	0/1

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